Novel stool-based protein biomarkers for improved colorectal cancer screening

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The MEDOCC plan

**WP1 – Biomarker based Screening**
- Better assays
  - Increase analytical sensitivity for DNA methylation assays
  - Improved DNA extraction
  - Nanotechnology based methylation detection
- Better markers
  - Protein markers*
  - Clinical validation & selection
  - Antibody based assay development
*separate funding

**WP2 – ctDNA liquid biopsy for residual disease detection**
- Better assays
  - ctDNA
  - Rearrangements
  - Mutations
  - Promoter methylation
- Observational study
  - Within PICNIC registry
  - Prospective observational design
  - Tissue & plasma collection
  - Repeated ctDNA assays
  - Clinical follow up compare to Wnt target gene methylation in tissue*
*separate funding

**WP3 - Turning into practice**
- Prospective screening trial
  - in the context of the Dutch CRC population based screening program with a paired design
  - Compare biomarker test to state of the art test (= FIT)
- Patient follow up
  - Correlate ctDNA & tissue methylation markers to:
    - Disease free survival at 3 years
    - Overall survival at 5 and 7 years

**Deliverables**
1) Better CRC stool-based screening tests ready to be implemented in CRC screening programs
2) Validated ctDNA liquid biopsy for residual disease detection & monitoring assay ready for intervention trial
3) Validated Wnt target gene methylation assay as prognostic marker, ready for intervention trial
4) Direct comparison of the prognostics value of liquid biopsy versus tissue based biomarker
CRC screening in the Netherlands

- Started in 2014
- All persons 55-75 year: biennial FIT
- Expected number of lives saved yearly 2400

Fecal immunochemical test (FIT) + Colonoscopy

FIT performance at specificity ~95%*

Sensitivity:
- CRC: ~65%
- Precursor lesions (advanced adenomas): ~27%

Room for improvement!!

Molecular changes as biomarkers in stool
Workflow: MS-based protein identification in stool

Sample series 1
N=22

Sample series 2
N=291

4-protein marker panels
By logistic regression and classification and regression tree (CART) analysis
Hemoglobin: sensitivity of 43% for detection of CRC at 95% specificity.
Marker panel: sensitivity of 71% versus 43% Hemoglobin alone at 95% specificity.
Hemoglobin: sensitivity of 8% for detection of advanced adenomas at 95% specificity.
Marker panel: Sensitivity of **40%** versus **8%** Hemoglobin alone at 95% specificity.
Does it work in a small FIT sample?

FIT
~ 10 mg stool

Multiplexed antibody-based assay

• Off the shelf assay for 4 out of top 50 (NOT top 4) proteins

*Not part of the top list of 29
Does it work in a small FIT sample?

FIT
~ 10 mg stool

Multiplexed antibody-based assay

FIT samples (50 µl)
\[ n = 72 \]

- Off the shelf assay for 4 out of top 50 (NOT top 4) proteins
Validation in FIT samples

1. CRCs vs controls: $P=2.0 \times 10^{-08}$

2. CRCs vs controls: $P=1.1 \times 10^{-05}$

3. CRCs vs controls: $P=0.1$

4. CRCs vs controls: $P=6.9 \times 10^{-05}$
Assay development for 10 selected candidates

*Outsource assay development

GLP / CLIA compliant quality

Meso Scale Discovery
Prospective cross-sectional screening trial within the Dutch CRC screening program

n= 10,000

FIT and biomarker test

Total number of individuals invited for FIT screening N=10,000

Number of individuals adhering to FIT screening N=6,000

FIT positive N=330 (5.5%)

Biomarker positive N=66 (20%)

Biomarker negative N=264 (80%)

Refer to colonoscopy
Detected AA + CRC N=156 (2.6%)
Prospective cross-sectional screening trial within the Dutch CRC screening program

n= 10,000

biomarker test

Total number of individuals invited for FIT screening N=10,000

Number of individuals adhering to FIT screening N=6,000

FIT positive N=330 (5.5%)

Biomarker positive N=66 (20%)

Refer to colonoscopyDetected AA + CRC N=156 (2.6%)

Biomarker negative N=264 (80%)

FIT negative N= 5,670 (94.5%)

(FIT negative) Biomarker positive N=284 (5%)

Refer to colonoscopyDetected AA + CRC N=31 (20% extra compared to FIT positive only)

(FIT negative) Biomarker negative N=5386 (95%)

No follow-up
Protein markers

Retrospective stool collections

Marker identification

Marker validation

Assay development

Prospective stool collections

Robust technology & automation

Prospective validation

Cost effectiveness

Clinical trial

Test approval & implementation

Dutch screening program from 2014
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Validation FIT sample set for Phase 1

**FF samples: Referral population n=177**

- Control: 93
- Adenomas: 32
- Advanced adenomas: 27
- CRCs: 25

**Cocos samples: CRC screening study n=1049**

- Control: 716
- Adenomas: 202
- AA/ASP: 123
- CRCs: 8

**FKG samples: Referral population n=72**

- Control: 24
- Adenomas: 18
- Advanced adenomas: 16
- CRCs: 14

**Total FITs @ AVL/NKI**

- Control: 833
- Adenomas: 252
- Advanced adenomas: 166
- CRCs: 47

Already aliquoted!